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Looking for relationships between the populations of *Dothistroma septosporum* in northern Europe and Asia

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A R T I C L E I N F O

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A B S T R A C T

Dothistroma septosporum, a notorious pine needle pathogen with an unknown historical geographic origin and poorly known distribution pathways, is nowadays found almost in all areas inhabited by pines (*Pinus* spp.). The main aim of this study was to determine the relationship between North European and East Asian populations. In total, 238 Eurasian *D. septosporum* isolates from 11 countries, including 211 isolates from northern Europe, 16 isolates from Russian Far East and 11 isolates from Bhutan were analysed using 11 species-specific microsatellite and mating type markers. The most diverse populations were found in northern Europe, including the Baltic countries, Finland and European Russia. Notably, *D. septosporum* has not caused heavy damage to *P. sylvestris* in northern Europe, which may suggest a long co-existence of the host and the pathogen. No indication was obtained that the Russian Far East or Bhutan could be the indigenous area of *D. septosporum*, as the genetic diversity of the fungus there was low and evidence suggests gene flow from northern Europe to Russian Far East. On the western coast of Norway, a unique genetic pattern was observed, which differed from haplotypes dominating other Fennoscandian populations. As an agent of dothistroma needle blight, only *D. septosporum* was documented in northern Europe and Asia, while *D. pini* was found in Ukraine and Serbia.

1. Introduction

Dothistroma needle blight (DNB) is a pine needle disease with a global distribution (Drenkhan et al., 2016). There are two causative agents of the disease: *Dothistroma septosporum* (Doroguine) M. Morelet and *D. pini* Hulbary (Barnes et al., 2004, 2016). DNB is in many geographic regions the most economically important needle disease of pines (Barnes et al., 2004), causing needle loss, growth retardation and even death of trees (Brown and Webber, 2008). For example, during the 2000s, DNB caused an annual loss of up to 19.8 million NZD to the economy of New Zealand (Watt et al., 2011a). Planted forest stands, arboreta, botanical gardens and parks are more damaged by *Dothistroma* spp. than areas where pines are native (Drenkhan et al., 2016). This may explain the higher disease severity in the southern hemisphere, where pines are introduced for plantation purposes. Currently > 100 host taxa are known to be susceptible to *Dothistroma* spp.

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(Drenkhan et al., 2016).

Dothistroma septosporum (as *Cytosporina septospora*) was first described by Doroguine (1911) in Saint Petersburg, Russia. Symptoms of DNB have also been observed in herbarium specimens collected in 1880 in Denmark (Munk, 1957) and in 1914 in Ukraine (Barnes et al., 2004). Almost a hundred years after the first description of *D. septosporum* in north-western Russia (Doroguine, 1911), the pathogen was noted in neighbouring Baltic and Nordic countries (Drenkhan and Hanso, 2009; Hanso and Drenkhan, 2008; Jovaišienė and Pavilionis, 2005; Markovskaja and Treigienė, 2009; Millberg et al., 2016; Müller et al., 2009; Solheim and Vuorinen, 2011). Currently the pathogen has a presence in almost all countries in Europe (Drenkhan et al., 2016). The origin and spread of the pathogen is under much investigation and recently, many studies using mostly microsatellite markers, have tried to elucidate these patterns of movement and spread between and within countries.

The population genetics of *D. septosporum* is relatively well investigated in certain parts of Europe, North America, South America, Africa and Oceania (Barnes et al., 2014; Drenkhan et al., 2013, 2016; Mullett et al., 2015, 2017). In central European countries, for example, the population characteristics and mating types of *D. septosporum* have been well documented and highlight various scenarios from populations that have been introduced by anthropogenic means to those that display characteristics typical of indigenous pathogens in native environments (Barnes et al., 2014; Boroń et al., 2016; Burgess and Wingfield, 2016; Drenkhan et al., 2013; Mullett et al., 2015, 2017; Ondrušková et al., 2017; Tomšovský et al., 2013). A population study of *D. septosporum* isolates from Czech Republic, Estonia and Finland showed low migration of the fungus from central to northern Europe (Drenkhan et al., 2013). In north-west Russia and some other east European countries, only mating type studies of *D. septosporum* have been carried out (Drenkhan et al., 2016). Furthermore, other population genetic studies have included only a low number of samples from Fennoscandia and no data from the Baltic countries or western Russia (Barnes et al., 2014; Mullett et al., 2015; Tomšovský et al., 2013). Little is known regarding the populations in northern and eastern Europe, where the oldest occurrences of the pathogen have been recorded. Thus, additional population studies are needed to understand the diversity and origin of the fungus in northern latitudes of Eurasia.

It has been speculated that *Dothistroma* spp. may originate from the Himalayas in Asia due to the low occurrence of DNB on native pines in indigenous areas (Ivory, 1994). DNB was first observed in Japan in 1952 (Ito et al., 1975). Later, the disease was found in India (Bakshi and Singh, 1968), Brunei (Peregrine, 1972) and Nepal (Ivory, 1990). Currently, there are reports of DNB from 12 Asian countries but the identity of the pathogen, as *D. septosporum*, has only been confirmed in one country (Drenkhan et al., 2016). *Dothistroma pini* has not yet been reported from Asia. In addition, the only genetic population study on Asian *D. septosporum* included a limited sample size of 12 isolates from Bhutan that showed high levels of diversity (Barnes et al., 2014). Knowledge of the genetic structure and diversity of additional populations from these countries, where species of pines are different to those native in Europe, may shed light on the potential geographic origin of this pathogen (McDonald, 1997; McDonald and Linde, 2002). Many highly damaging forest pathogens are of non-native or unknown origins (Wingfield et al., 2015). Information on their origins is essential to elucidate pathways of introduction and spread as well as improving quarantine regulations and biosecurity measures.

Several population studies provide data supporting Europe as the origin of *D. septosporum* (Barnes et al., 2014; Drenkhan et al., 2013; Mullett et al., 2017; Piotrowska et al., 2017). However, the recent increase in recordings of the pathogen in Europe could be due to climate change, where models predict a further increase in area suitable for DNB in northern Europe under climate change projections (Watt et al., 2011b). Alternatively, new genotypes of the pathogen may have emerged and spread naturally or via anthropogenic activity to northern Europe from Asia. Anthropogenic activity and international trade have been acknowledged as the most significant contributors to the dispersal of pathogens to new environments (Barnes et al., 2014; Wingfield et al., 2015).

A dispersal route of *Dothistroma septosporum* from East Asia to Europe is possible as many conifer species, including *Pinus koraiensis* Sieb. et Zucc., *P. pumila* Regel, and *P. sibirica* (Rupr.) have been introduced from that region to Europe via trade since the 19th century (Drenkhan et al., 2014). It is unknown to what extent conifer trees have been exported from Europe to East Asia. Natural dispersal of the fungus between northern Europe and Russian Far East could also be considered as possible, since the ranges of different *Pinus* species cover a continuous Eurasian forest belt from the Atlantic to the Pacific Ocean. Many *Pinus* species of this belt, including *P. densiflora* Sieb. et Zucc., *P. koraiensis, P. sibirica* and *P. sylvestris* are susceptible to DNB (see Drenkhan et al., 2016).

The objective of this study was to determine if patterns of spread exist between northern Europe and Asian populations based on natural dispersal events or anthropogenic movement of the pathogen. In addition, to determine if the natural range of *P. sylvestris* had any influence on the population structure and diversity of the pathogen. More precisely, the aims of this study were, therefore, to (i) determine whether *D. septosporum* and/or *D. pini* is the causal agent of DNB in northern Europe and Asia, (ii) to determine the diversity and population structure of northern European and some Asian populations of *D. septosporum,* and test whether migration of *D. septosporum* has occurred between these areas, (iii) and to test the occurrence of sexual recombination among populations by determining the mating type frequency. A combination of previously developed microsatellite markers (Barnes et al., 2008) and species-specific mating type markers (Groenewald et al., 2007) were used for this study.

2. Materials and methods

2.1. Sample collection, fungal isolation, DNA extraction and identification

Pine needles with DNB symptoms were collected from one or several locations from 10 countries in Europe and from Bhutan and Russian Far East in Asia (Table 1; Fig. 1). Sampled host species included 11 different *Pinus* species (Table 1). The isolates from Bhutan were the same as those in Barnes et al. (2014).

In Estonia, Finland, Latvia, Lithuania, Norway, Russia, Serbia, Sweden and Ukraine the samples were collected from 6- to 30-year-old *Pinus* trees. The distance between sampled trees within these sites was at least 5–20 m. Swedish isolates from *P. contorta* Douglas ex Loudon seedlings (about 6-year-old) were obtained from plants growing 10 cm from each other. All sampled needles were collected from the lower parts of crowns and only one isolate per sampled tree was used in the analyses.

Geographically close sample locations in the same country that were not separated by natural barriers are referred to as sampling sites (Table 1). Seventeen sampling sites in Eurasia were considered for analyses (Table 1).

Fungal isolations were performed according to the protocols of Mullett and Barnes (2012). Approximately 0.04 g of mycelium from the colony edge was transferred into 2.0 ml micro centrifuge tubes and stored at −20 °C for DNA extraction. Mycelium was homogenized with a Retsch MM400 homogenizer (Retsch GmbH, Haan, Germany) using a mix of quartz sand (Scharlab S.L., Spain) and metal beads (∅ 2.5 mm). DNA was extracted using the E.Z.N.A Fungal DNA Mini Kit (Omega Bio-Tek Inc., Norcross, GA, USA).

To determine the identity of the isolates, species-specific mating type primers were used according to Groenewald et al. (2007) (see Section 2.4). For isolates that screened positive for *D. pini*, their identity was confirmed by performing a second round of PCR using species-specific primers DPtef-F and DPtef-R (Ioos et al., 2010). PCR reactions

Table 1
Dothistroma septosporum isolates investigated in this study.

Sampling sites code[a]	Sampling location	Geographical coordinates[b]		Host species	No. of isolates	Sampling date	Collector(s)
		N	E				
DEN		**55.88731**	**12.48753**		**11**		
	Hørsholm	55.86597	12.50960	*P. ponderosa* Dougl. ex Laws.	4	Oct. 2013	A. Kačergius, S. Markovskaja, I. Barnes, I. M. Thomsen, M. S. Mullett
	Hørsholm	55.86597	12.50960	*P. aristata* Engelm.	1	Oct. 2013	I. Barnes
	Hørsholm	55.86597	12.50960	*P. attenuata* Lemm.	3	Oct. 2013	I. Barnes, I. M. Thomsen, M. S. Mullett
	Hørsholm	55.86597	12.50960	*P. contorta* Doug. ex Loud.	1	Oct. 2013	M. S. Mullett
	Fredensborg	55.98327	12.38791	*P. sylvestris* L.	2	Oct. 2013	M. S. Mullett
EST1	Konguta	**58.22786**	**26.15673**	*P. sylvestris*	15	Oct. 2007-June 2008; Aug. 2013	R. Drenkhan
EST2	Kautsi	**57.70618**	**26.58521**	*P. sylvestris*	25	Dec. 2007-March 2008; Oct. 2012; Feb. 2013	R. Drenkhan
FIN		**62.65036**	**27.05760**		**18**		
	Jormala	62.55620	27.34930	*P. sylvestris*	3	Aug. 2011	I. Barnes
	Kökkö	61.49741	27.35077	*P. sylvestris*	1	Oct. 2014	M. Vuorinen
	Pyhtään	60.50895	26.55697	*P. sylvestris*	1	Nov. 2014	M. Vuorinen
	Rautalampi	62.65364	26.66816	*P. sylvestris*	1	March 2014	M. Vuorinen
	Suonenjoki	62.63700	27.05916	*P. sylvestris*	11	April-June 2008; Oct. 2014	M. Vuorinen, M. M. Müller
	Vanttauskoski	66.36637	26.73442	*P. sylvestris*	1	June 2014	M. Vuorinen
LAT		**56.80740**	**24.97695**		**20**		
	Kegums	56.73768	24.74188	*P. sylvestris*	18	Nov. 2013	R. D. Priedite
	Skujas	57.41237	27.13346	*P. sylvestris*	2	June 2013	R. D. Priedite
LIT		**54.63949**	**24.89050**		**12**		
	Aukštadvaris	54.57620	24.51527	*P. sylvestris*	1	Nov. 2012	A. Kačergius, S. Markovskaja
	Bajorai	54.76344	25.30631	*P. sylvestris*	2	Oct. 2012	A. Kačergius, S. Markovskaja
	Baltoji Voké	54.45261	25.11779	*P. sylvestris*	2	May 2013	A. Kačergius, S. Markovskaja
	Marcinkonys	54.06231	24.42894	*P. sylvestris*	1	May 2012	A. Kačergius, S. Markovskaja
	Miškonys	54.79885	25.39677	*P. sylvestris*	2	Nov. 2013	A. Kačergius, S. Markovskaja
	Paliuliškés	54.73462	25.40583	*P. nigra* Arnold	1	May 2013	A. Kačergius, S. Markovskaja
	Petrašiškés	54.57667	24.57603	*P. sylvestris*	1	Oct. 2012	A. Kačergius, S. Markovskaja
	Vaišvydava	54.84946	24.03536	*P. nigra*	1	Oct. 2013	A. Kačergius, S. Markovskaja
	Vaišvydava	54.84959	24.03851	*P. peuce* Griseb.	1	Oct. 2013	A. Kačergius, S. Markovskaja
NOR1		**61.22056**	**11.54273**		**21**		
	Engerdal	61.74612	11.97591	*P. sylvestris*	1	April 2010	H. Solheim
	Engerdal	61.75198	11.97206	*P. sylvestris*	10	July 2013; March-April 2015	H. Solheim
	Gransherad	59.69179	9.04286	*P. sylvestris*	1	July 2014	H. Solheim
	Tufte	59.24659	9.58472	*P. sylvestris*	1	Nov. 2011	H. Solheim
	Konnerud	59.72234	10.06238	*P. sylvestris*	1	June 2010	H. Solheim, M. Vuorinen
	Koppang	61.59182	10.99175	*P. sylvestris*	1	Aug. 2012	H. Solheim
	Nybergsund	61.24555	12.34866	*P. sylvestris*	2	June 2010	H. Solheim
	Nybergsund	61.25245	12.33995	*P. sylvestris*	1	June 2010	H. Solheim
	Skotterud	59.99248	12.10700	*P. sylvestris*	2	May 2010	H. Solheim, M. Vuorinen
	Telneset	62.35100	10.88284	*P. sylvestris*	1	Aug. 2012	H. Solheim
NOR2		**61.46869**	**5.59441**		**9**		
	Naustdal	61.46810	5.58838	*P. sylvestris*	4	June 2013	H. Solheim
	Naustdal	61.46914	5.59922	*P. sylvestris*	5	June 2013	H. Solheim
NOR3	Ulvedal	**61.83943**	**6.48750**	*P. sylvestris*	**22**	July 2013; Aug. 2014	H. Solheim
NOR4		**69.00600**	**18.61035**		**4**		
	Tune	68.93063	18.47570	*P. sylvestris*	1	June 2011	H. Solheim
	Målselvfossen	69.03110	18.65543	*P. sylvestris*	3	June 2011	H. Solheim
SER	Troglan Bara	**43.96166**	**21.72027**	*P. nigra*	**9**	Sept. 2013	N. Keča
SWE		**59.97168**	**15.73122**		**4**		
	Fagersta	59.97168	15.73122	*P. sylvestris*	2	May 2015	H. Millberg
	Fagersta	59.97168	15.73122	*P. contorta*	2	May 2015	H. Millberg
UKR		**49.40091**	**35.59201**		**14**		
	Petrivka	49.40091	35.59201	*P. sylvestris*	3	Dec. 2013	K. Davydenko
	Petrivka	49.40091	35.59201	*P. nigra* subsp. *pallasiana* (Lamb) Holmb.	11	Dec. 2013	K. Davydenko
WRUS		**60.35779**	**29.95791**		**27**		
	Kameshki	60.20060	29.96010	*P. sylvestris*	13	Nov. 2013	R. Drenkhan, D. L. Musolin
	Ruskeala	61.94539	30.57953	*P. sylvestris*	2	Aug. 2014	K. Ligi
	Simagino	60.28500	29.81200	*P. sylvestris*	11	Nov. 2013	R. Drenkhan, D. L. Musolin
	St. Petersburg	60.02278	30.35167	*P. sylvestris*	1	Nov. 2013	R. Drenkhan, D. L. Musolin
BHU		**27.50136**	**90.52556**		**11**		
	Yusipang	27.46440	89.70706	*P. radiata* D. Don.	3	July 2005	T. Kirisits, M. J. Wingfield, D. B. Chhetri
	URA	27.48534	90.90804	*P. wallichiana* Jacks.	1	May 2005	H. Konrad, D. B. Chhetri

(*continued on next page*)

Table 1 (*continued*)

Sampling sites code[a]	Sampling location	Geographical coordinates[b]		Host species	No. of isolates	Sampling date	Collector(s)
		N	E				
	Tangsibi	27.50717	90.86065	*P. wallichiana*	5	June 2005	T. Kirisits, M. J. Wingfield, D. B. Chhetri
	Lamey Goemba	27.54529	90.72478	*P. wallichiana*	2	July 2005	T. Kirisits, N. Gyeltshen
F-EAST1		**44.11270**	**132.72435**		**8**		
	Primorskiy Kray, Nikolaevka	43.86178	132.45480	*P. densiflora* Sieb. et Zucc.	2	Aug. 2014	R. Drenkhan, H. Solheim
	Primorskiy Kray, Rettikhovka	44.17533	132.79210	*P. sylvestris*	6	Aug. 2014	R. Drenkhan, H. Solheim
F-EAST2		**48.34435**	**135.19741**		**8**		
	Khabarovskiy Kray, Nekrasovka	48.33415	135.27478	*P. sylvestris*	1	Aug. 2014	R. Drenkhan, H. Solheim
	Khabarovskiy Kray, Nekrasovka	48.34580	135.18635	*P. sylvestris*	7	Aug. 2014	R. Drenkhan, H. Solheim

[a] Sampling sites codes: BHU – Bhutan, DEN – Denmark, EST – Estonia, FIN – Finland, F-EAST – Russian Far East, LAT – Latvia, LIT – Lithuania, NOR – Norway, SER – Serbia, SWE – Sweden, UKR – Ukraine, WRUS – western Russia.

[b] The coordinates of the sampling sites (in bold) are based on the weighted midpoints of sampling locations. Within the sampling sites the maximum distance between sampling locations is: **BHU** – up to 119 km; **DEN** –15 km; **EST1** – 0.1 km; **EST2** – 0.3 km; **FIN** – 651 km; **F-EAST1** – 44 km; **FEAST2** – 6.6 km; **LAT** –163 km; **LIT** – 103 km; **NOR1** – 352 km; **NOR2** – 0.6 km; **NOR3** – 0.1 km; **NOR4** – 13 km; **SER** – 0.1 km; **SWE** – 0.1 km; **UKR** – 0.1 km; **WRUS** – 214 km.

were carried out in 20 µl volumes following the protocol of Ioos et al. (2010) with some modifications: 2 µl DNA template, a final concentration of 0.4 µM of each forward and reverse primers, 0.2 mM dNTP mix (Solis BioDyne, Tartu, Estonia), 0.05U polymerase (Solis BioDyne), 2 µl of 10x Buffer B1 (HOT FIREPol, Solis BioDyne) and 2 mM $MgCl_2$. Cycling conditions followed those of Ioos et al. (2010). Positive amplification using these primers confirms the presence of *D. pini*.

All PCRs were carried out on a TProfessional Thermocycler (Biometra, Göttingen, Germany). PCR products were visualized on 1% agarose gel (SeaKem® LE Agarose, Lonza) under UV light using a Quantum ST4-system (VilberLourmat SAS, Marne-la-Vallée, France).

2.2. Haplotype determination

For multilocus haplotyping, 11 microsatellite markers were used: Doth_DS1, Doth_DS2, Doth_I, Doth_E, Doth_F, Doth_G, Doth_J, Doth_O, Doth_M, Doth_K and Doth_L (Barnes et al., 2008). The forward primers of each pair were fluorescently labelled following Mullett et al. (2015). PCR reactions were performed in 25 µl reaction volumes, consisting of 1 µl template DNA, a final concentration of 0.3 µM forward and reverse primer, 0.2 mM dNTP mix (Promega Corporation, Sweden), 5 µl 5x GoTaq Clear Buffer (Promega Corporation), 1.5 mM $MgCl_2$ (Promega Corporation), 1 U Taq Polymerase (Promega Corporation) and 10.8 µl PCR grade water. PCR was done as described by Barnes et al. (2008).

PCR products for fragment analysis were pooled into two separate panels as outlined in Mullett et al. (2015). Panels of PCR products were analysed using an Applied Biosystems 3130XL genetic analyser along with LIZ 600 size standard (Applied Biosystems) and alleles scored using GENEMAPPER v5.0 (Applied Biosystems, Carlsbad, USA).

2.3. Genetic diversity and differentiation of populations

Individuals with identical alleles at all microsatellite loci were considered clones. Two datasets were generated: one containing all individuals, i.e. the non-clone-corrected (non-cc) dataset; and one containing only one multilocus haplotype per population/sampling site, i.e. the clone-corrected dataset (cc). The non-cc dataset was used to calculate the total number of haplotypes using GENALEX 6.5 (Peakall

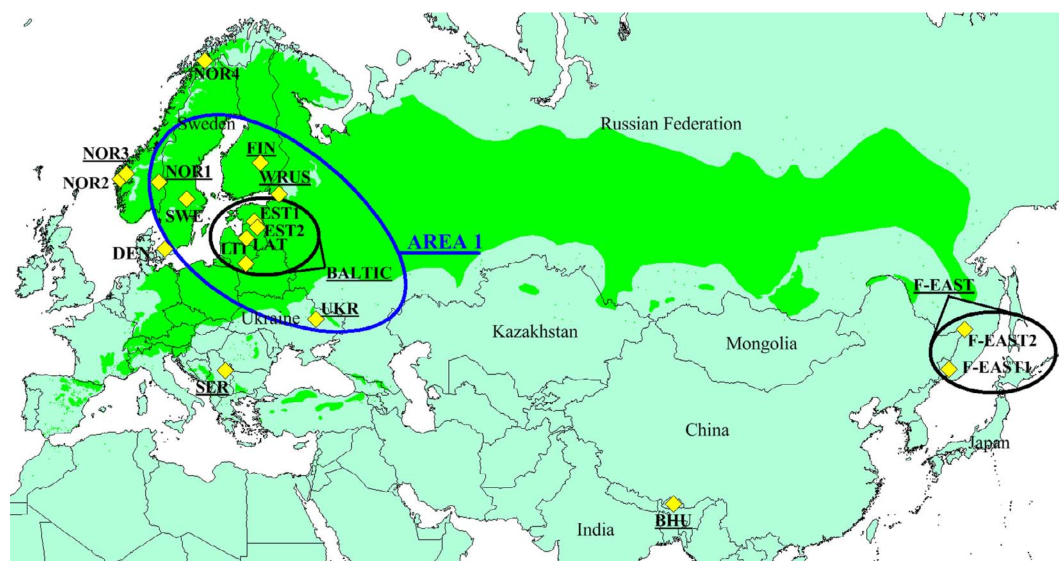

Fig. 1. Map of the sampling sites of *Dothistroma septosporum*. Yellow symbols indicate the weighted geographical midpoint of the 17 sampling sites (Table 1). Black circles and underlined sampling site populations are referred to as the 9 merged populations. The bright green area indicates the natural distribution area of *Pinus sylvestris* in Eurasia (source: http://www.euforgen.org/species/pinus-sylvestris/). The blue circle indicates the most diverse region, i.e. including diverse populations with low genetic distance from each other (Area 1). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and Smouse, 2012), the mating type ratios and the linkage disequilibrium index (I_A) (see Section 2.4). Both cc and non-cc data sets were used for mating tests to reduce the chance of rejecting the null hypothesis of random mating that a smaller clone-corrected data set might carry (Milgroom, 1996). The clone-corrected dataset was used to calculate mean haploid genetic diversity (h), total number of alleles, unique alleles, mean number of different alleles, and mean unbiased diversity (uh) for each population using GENALEX 6.5. Allelic richness (A_R, the number of distinct alleles in a sampling site) and private allelic richness (PA_R, the number of alleles unique to a particular sampling site), were calculated with ADZE 1.0 using the rarefaction approach, with population sizes standardized to the smallest sample size, which allows comparison of populations with different sample sizes (Szpiech et al., 2008).

An analysis of molecular variance (AMOVA) on the cc dataset was used to test for significant differentiation between the 17 sampling sites (see Table S1). Sampling sites that did not differ significantly from each other ($p > 0.05$) were grouped and treated as a single population. Due to the very low number of isolates (N (cc) ≤ 4, see Table S1) DEN, NOR4 and SWE sampling site populations were not merged with other populations. Sampling sites with fewer than nine multilocus haplotypes (DEN, NOR2, NOR4 and SWE) were removed from further calculations of diversity indices (Table 2) but were included in the mating type determination.

2.4. Mating type determination and random mating

Mating types of the *D. septosporum* isolates were determined with the species-specific mating type primers of Groenewald et al. (2007), which also served to confirm the isolates identity as *D. septosporum*. The 20 µl PCR reaction mix consisted of 4 µl of 5 × HOT FIREPol Blend Master Mix with BSA with 7.5 mM $MgCl_2$ (Solis BioDyne), a final concentration of 0.5 µM each mating type primers: DseptoMat1f, DotMat1r and DseptoMat2f, DotMat2r (Groenewald et al., 2007), 1 µl template DNA and 13 µl PCR grade water. PCR conditions were carried out as described in Groenewald et al. (2007), with the adjustment of the initial denaturation step to 95 °C for 12 min.

In order to investigate the possibility of sexual recombination, two tests were carried out on both cc and non-cc datasets for populations with over five isolates. Firstly, an exact binomial test, using two-tailed P-values, was used to test whether mating type ratios deviated from a 1:1 ratio that shows evidence of random mating. Secondly, the index of association (I_A) was used to test for haploid linkage disequilibrium of the 11 microsatellite alleles in Genalex 6.5 (Peakall and Smouse, 2012).

2.5. Isolation by distance

Native, or well established populations that spread naturally across the landscape, are characterized by isolation by distance where geographically close individuals are also more genetically related. As geographic distance increases between individuals, so does genetic distance. Introduced populations generally lack isolation by distance

Table 2
Diversity statistics of *D. septosporum* for 17 sampling sites (*) and nine merged populations (bold), based on 11 microsatellite markers.

Sampling site and/or population code	N non-cc[a]	No. of haplotypes cc[b]	% of different haplotypes non-cc[a]	Mean haploid genetic diversity h (SE)[c] cc[b]	Total no. of alleles cc[b]	Unique alleles cc[b]	Mean allelic richness A_R[d] (SE)[c] cc[b]	Mean private allelic richness P_{AR}[d] (SE)[c] cc[b]	Mean number of different alleles Na (SE)[c] cc[b]	Mean unbiased diversity uh (SE)[c] cc[b]
BALTIC[e]	**72**	**72**	**100**	**0.69 (0.08)**	**120**	**31**	**4.23 (0.59)**	**0.59 (0.25)**	**10.91 (2.57)**	**0.70 (0.08)**
EST1*	15	15	100		63	3				
EST2*	25	25	100		76	10				
LAT*	20	20	100		67	4				
LIT*	12	12	100		63	3				
FIN*	**18**	**18**	**100**	**0.61 (0.08)**	**64**	**9**	**3.78 (0.52)**	**0.57 (0.23)**	**5.82 (0.97)**	**0.65 (0.08)**
NOR1*	**21**	**18**	**86**	**0.60 (0.08)**	**53**	**5**	**3.60 (0.52)**	**0.59 (0.29)**	**4.82 (0.80)**	**0.64 (0.09)**
NOR3*	**22**	**15**	**68**	**0.51 (0.06)**	**39**	**2**	**3.06 (0.36)**	**0.47 (0.29)**	**3.55 (0.43)**	**0.55 (0.07)**
SER*	**9**	**9**	**100**	**0.43 (0.08)**	**31**	**2**	**2.73 (0.40)**	**0.53 (0.21)**	**2.82 (0.38)**	**0.49 (0.10)**
UKR*	**14**	**14**	**100**	**0.58 (0.05)**	**48**	**2**	**3.25 (0.37)**	**0.50 (0.31)**	**4.36 (0.74)**	**0.62 (0.06)**
WRUS*	**27**	**21**	**78**	**0.66 (0.06)**	**66**	**2**	**4.00 (0.49)**	**0.42 (0.15)**	**6.00 (0.92)**	**0.70 (0.07)**
BHU*	**11**	**10**	**91**	**0.38 (0.10)**	**34**	**5**	**2.71 (0.53)**	**0.52 (0.22)**	**3.09 (0.60)**	**0.42 (0.11)**
F-EAST[f]	**16**	**13**	**81**	**0.49 (0.07)**	**40**	**4**	**2.90 (0.38)**	**0.43 (0.25)**	**3.64 (0.54)**	**0.54 (0.08)**
F-EAST1*	8	7	88		31	3				
F-EAST2*	8	6	75		27	1				
DEN[g]	*11*	*4*	*36*		*25*	*1*				
NOR2[g]*	*9*	*5*	*56*		*20*	*0*				
NOR4[g]*	*4*	*4*	*100*		*18*	*0*				
SWE[g]*	*4*	*4*	*100*		*31*	*2*				
All Europe[h]	211	184	87		160	42				
Europe[i]	**183**	**167**	**91**	**0.71 (0.07)**	**157**	**62**	**4.62 (0.59)**	**2.61 (0.59)**	**14.27 (3.84)**	**0.71 (0.07)**
Asia	**27**	**23**	**85**	**0.56 (0.07)**	**60**	**9**	**3.52 (0.42)**	**1.51 (0.39)**	**5.46 (0.82)**	**0.58 (0.07)**
All data[j]	238	207	87		179	51				
All populations[k]	**210**	**190**	**90**	**0.55 (0.03)**	**166**	**71**	**4.58 (0.59)**	**4.58 (0.53)**	**5.00 (0.42)**	**0.59 (0.03)**

[a] non-cc = non-clone-corrected dataset.
[b] cc = clone-corrected dataset.
[c] SE = standard error.
[d] Calculations were standardized to a population size N(cc) = 9.
[e] Based on the results from the AMOVA, the Baltic isolates (EST1, EST2, LAT and LIT) were combined into a single population BALTIC.
[f] Based on the results from the AMOVA, the Russian Far East isolates (F-EAST1 and 2) were combined into a single population F-EAST.
[g] Due to small sample size after clone correction, (N < 9), these populations were excluded from future population genetic analyses.
[h] 14 European sampling sites.
[i] 7 merged European populations (N(cc) ≥ 9).
[j] All 17 Eurasian sampling sites.
[k] 9 merged Eurasian populations (N(cc) ≥ 9).

due to genetic bottlenecks and explosive, often clonal, spread. Mantel tests, conducted in Genalex 6.5, were used to test for isolation by distance on the cc dataset using Nei's genetic distance (Nei, 1972, 1978) and geographic distances. Two tests were carried out: one contained all nine merged populations (Table 2) and the other tested only the seven merged European populations.

For visualization of Nei's genetic distances, Principal Coordinates Analysis (PCoA) was carried out in GENALEX 6.5 using the covariance standardized method on the cc dataset of the nine merged populations.

2.6. Population clustering

To assign individuals into populations and determine structure within populations, without any a priori knowledge (i.e. geographic origin, host) the Bayesian programme STRUCTURE (Falush et al., 2003) was used. Each of 20 independent runs of K = 1–20 were carried out with 100,000 burn-in iterations followed by a run of 500,000. The optimum number of clusters (K) was determined using the ln(Pr(X|K)) method (Pritchard et al., 2000, 2010) in CLUMPAK (Kopelman et al., 2015). The final assignment of individuals to clusters was carried out on the optimum K by applying 100,000 burn-in iterations, followed by 1,000,000 runs.

2.7. Migration between populations

To detect gene flow between population pairs, MIGRATE-N 3.6 (Beerli, 2009, 2010; Beerli and Palczewski, 2010) was used on the cc dataset. The programme uses the Likelihood/Bayesian method and Markov Chain Monte Carlo simulations to calculate immigration rates (M = m/mμ) between the populations and number of immigrants per generation (ΘM). Microsatellite input data was generated by MICROSATELLITE ANALYZER 4.05 (Dieringer and Schlötterer, 2003) and the single step microsatellite model was used. For the start parameters, estimates with FST (Fw/Fb) measure for a simple estimate of theta and migration rate were used, while for the FST calculation, the variable theta and M symmetric method was used. Analyses were performed using one long chain and 5000 steps were recorded after every 100 steps with a burn-in of 10,000. Migration between all populations was also tested for and additional analyses were conducted to determine the migration scale between Asia and Europe, between south-central and eastern Asia and lastly, all European populations were merged as well as the eastern Asian ones. In MIGRATE-N, arithmetic averages of mutation-scaled immigration rates were calculated for all population pairs and were then used to determine the relative significance of the values. Only when values were above the arithmetic average of the analysed populations, was significant migration considered to have occurred.

3. Results

3.1. Identification of Dothistroma spp

The majority of isolates (N = 238) in this study were identified as *D. septosporum*. *Dothistroma pini* isolates (N = 15) were found in Ukraine on *P. nigra* subsp. *pallasiana* (Lamb.) Holmb. (geographical locations: 46.60193°N, 32.81324°E; 46.49745°N, 32.53258°E; 46.53005°N, 31.60417°E) and in Serbia (N = 17) on *P. nigra* Arnold (44.94167°N, 21.10944°E). In northern Europe and Asia only *D. septosporum* was detected. *Dothistroma pini* isolates were excluded from all further analyses.

3.2. Multilocus haplotypes and sampling sites of D. septosporum

A total of 238 Eurasian *D. septosporum* isolates from 11 countries comprising 17 sampling sites were analysed (Table 1, Fig. 1). Analyses across 11 microsatellite markers resulted in a total of 179 alleles (see Table S2).

In total, 207 different haplotypes were found among the 238 *D. septosporum* isolates investigated (Table 1). The highest percentage of different haplotypes was observed in nine sampling sites (EST1, EST2, FIN, LAT, LIT, NOR4, SER, SWE and UKR), where the number of haplotypes was equal to the number of analysed samples (i.e. no clones were found at these sites) (Table 2). Clones were observed within the DEN, NOR1, NOR2, NOR3, WRUS, F-EAST1, F-EAST2, and BHU sampling sites. No clones were shared between different sampling sites.

The AMOVA indicated similarity among the Baltic sampling sites (Table S1), i.e. EST1, EST2, LAT, and LIT, and did not show any evidence of population differentiation (p-values ranged from 0.108 to 0.465). These populations were, therefore, combined into a single population called BALTIC. The WRUS sampling site population was similar to LAT and LIT (p-values ranged from 0.065 to 0.145) but was significantly different from EST1 and EST2 ($p < 0.05$) and was therefore not merged with the BALTIC population (Table S1). The two Russian Far East sampling sites were also not significantly differentiated (p = 0.344) and were combined into a single population named F-EAST. Surprisingly the NOR2 and NOR3 populations, separated from each other by only *ca.* 60 km on the Atlantic coast of Norway, were significantly differentiated ($p < 0.05$). After the AMOVA grouping of the above sampling sites into populations and removal of four sampling sites (DEN, SWE, NOR2 and NOR4) due to low sample size (N (cc) < 9), nine Eurasian populations were retained for comparison: BALTIC, FIN, NOR1, NOR3, SER, UKR, WRUS, BHU and F-EAST (Table 2).

3.3. Genetic diversity and population statistics

The Baltic and western Russian populations were the most diverse in this study. They had the highest mean unbiased diversity values (uh = 0.7), the highest genetic diversity (h = 0.66–0.69), the highest number of different alleles (66–120), and the highest mean allelic richness (A_R = 4.00–4.23) (Table 2). The populations from FIN, NOR1 and UKR were the second highest in terms of genetic diversity. Populations from the BALTIC and NOR1 had the highest value of mean private allelic richness (P_{AR} = 0.59) while WRUS and F-EAST populations had the lowest values ($P_{AR} < 0.43$).

The group of populations with statistically higher than average diversity and lowest genetic distance between them is found in an area indicated as Area 1 (Fig. 1 and Table 3) and consists of the Baltic, Finland, western Russian, Norway (NOR1) and Ukrainian populations. These populations also form a geographically coherent group with genetic distances between populations ranging from 0.055 to 0.287. In Eurasia, as the geographical distance from the centre of this area increases, the diversity of the populations decreases. All *D. septosporum* populations in Area 1 remain within the native range of *P. sylvestris* (bright green shading in Fig. 1), however the population in Ukraine is on the edge of this range in a fragmented distribution islet of *P. sylvestris*.

All of the populations in Area 1 have statistically higher diversity indices than the Asian populations (BHU and F-EAST). The European populations are clearly more diverse than the Asian populations according to various diversity indices presented in Table 2. The Asian populations are located within the native distribution area of the genus *Pinus* (see Fig. S1) but not of *P. sylvestris* (Fig. 1).

Based on PCoA and Nei's genetic distance, the population in Serbia was genetically distinct from all the other analysed populations of *D. septosporum* in Europe and Asia (see Fig. 2, Table 3, shown in bold). Nei's genetic distance indicated that the population in Russian Far East was relatively close to populations in Europe, except the Serbian one, but distant from the other Asian population in Bhutan (Table 3). Furthermore, Nei's genetic distances between the Bhutan population and the populations in Baltics, Finland and western Russia (northern Europe) are smaller than between Bhutan and any other analysed populations (NOR1, NOR3, SER, UKR, F-EAST, see Table 3).

Table 3
Nei's genetic distances on the clone-corrected dataset and geographical distances between population pairs. Geographical distances are given in kilometres and are shown above the diagonal. Nei's genetic distances are below the diagonal. Genetic differences that are higher than arithmetic average values are given in bold. The lowest genetic distances between population pairs in Area 1 (Baltic, Finland, western Russia, Ukraine and Norway (NOR1)) are highlighted in blue.

	BALTIC	FIN	NOR1	NOR3	SER	UKR	WRUS	BHU	F-EAST
BALTIC	-	626	930	1205	1072	1483	440	5963	6754
FIN	0.091	-	825	1064	1563	2106	298	5970	6303
NOR1	0.160	0.120	-	277	1990	2033	1001	6772	7001
NOR3	0.377	0.369	0.383	-	2260	2222	1265	7030	7117
SER	**0.574**	**0.667**	**0.755**	**0.640**	-	1216	1269	5224	6795
UKR	0.148	0.287	0.266	**0.521**	**0.707**	-	1904	6287	7978
WRUS	0.059	0.055	0.104	**0.416**	**0.556**	0.242	-	5772	6334
BHU	0.331	0.272	**0.428**	**0.490**	**0.857**	**0.723**	0.375	-	4313
F-EAST	0.268	0.309	0.383	0.379	**0.585**	0.230	0.274	**0.476**	-

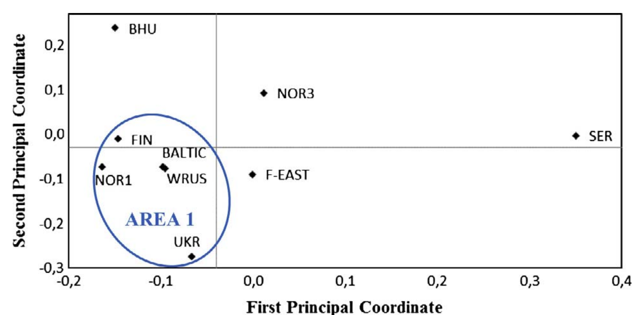

Fig. 2. Principal Coordinate Analysis of nine populations of *D. septosporum* (two axes show 53.1% of variation). Grouping of populations in Area 1 are based on the information from Fig. 1.

3.4. Mating type distribution and haploid linkage disequilibrium

Both mating type idiomorphs were found in all sampling sites, except in NOR4 and SWE. Where both mating types existed, they occurred at equal ratios in almost all populations ($p > 0.05$; see Table 4). Statistically significant ($p < 0.05$) unequal mating type ratios were recorded only in sampling sites EST2 (both cc and non-cc datasets), WRUS and DEN (non-cc dataset only). The *MAT1-1* idiomorph dominated the EST2 and WRUS populations, while the *MAT1-2* idiomorph dominated the DEN sampling site. Only the *MAT1-2* idiomorph was found in the NOR4 (the northernmost sampling site population in Norway) and SWE sampling sites.

The index of association indicated that random mating occurred in the Baltic and Ukraine populations, using both clone-corrected and non-clone-corrected datasets, and in Bhutan using only the clone-corrected dataset (Table 4). The index of association rejected random mating in all other merged populations. Additionally, random mating was found

Table 4
A summary of the mating type distribution and linkage disequilibrium statistics of 11 microsatellite markers of *D. septosporum* in the 17 sampling sites (*) and in the nine merged populations (bold).

Population code	*MAT1-1* non-cc[a]	*MAT1-2* non-cc[a]	P-value of exact binomial test non-cc[a]	*MAT1-1* cc[b]	*MAT1-2* cc[b]	P-value of exact binomial test cc[b]	I_A[c] non-cc[a]	P-value of I_A non-cc[a]	I_A[c] cc[b]	P-value of I_A[c] cc[b]
BALTIC[d]	**38**	**34**	**0.724**	**38**	**34**	**0.724**	**1.94**	**0.053**	**1.94**	**0.053**
EST1*	9	6	0.607	9	6	0.607	4.81	0.027	4.81	0.027
EST2*	18	7	0.043	18	7	0.043	3.15	0.312	3.15	0.312
LAT*	8	12	0.503	8	12	0.503	3.92	0.039	3.92	0.039
LIT*	3	9	0.146	3	9	0.146	5.53	0.047	5.53	0.047
FIN*	**11**	**7**	**0.481**	**11**	**7**	**0.481**	**3.74**	**0.001**	**3.74**	**0.001**
NOR1*	**13**	**8**	**0.383**	**11**	**7**	**0.481**	**4.38**	**0.001**	**4.40**	**0.001**
NOR3*	**8**	**14**	**0.286**	**6**	**9**	**0.607**	**5.80**	**0.001**	**5.03**	**0.001**
SER*	**5**	**4**	**1.000**	**5**	**4**	**1.000**	**4.72**	**0.007**	**4.72**	**0.007**
UKR*	**4**	**10**	**0.181**	**4**	**10**	**0.181**	**3.42**	**0.207**	**3.42**	**0.207**
WRUS*	**20**	**7**	**0.019**	**15**	**6**	**0.078**	**3.93**	**0.001**	**3.75**	**0.001**
BHU*	**4**	**7**	**0.549**	**4**	**6**	**0.754**	**3.29**	**0.039**	**3.42**	**0.133**
F-EAST[e]	**7**	**9**	**0.804**	**6**	**7**	**1.000**	**3.27**	**0.001**	**3.37**	**0.023**
F-EAST1*	5	3	0.727	4	3	1.000	4.72	0.008	4.36	0.129
F-EAST2*	2	6	0.289	2	4	0.688	5.11	0.001	5.09	0.029
DEN[f*]	*1*	*8*	*0.039*	*1*	*3*	*0.625*	*6.75*	*0.001*	*7.17*	*0.207*
NOR2[f*]	*4*	*5*	*1.000*	*2*	*3*	*1.000*	*7.42*	*0.001*	*7.03*	*0.004*
NOR4[f*]	*0*	*4*	–	*0*	*4*	–	–	–	–	–
SWE[6*]	*Nd*[g]	*2*	–	–	*2*	–	–	–	–	–

[a] non-cc = non-clone-corrected dataset.
[b] cc = clone-corrected dataset.
[c] I_A = index of association.
[d] According to the AMOVA, the Baltic isolates (EST1, EST2, LAT and LIT) were combined into a single population BALTIC.
[e] According to the AMOVA, the Russian Far East isolates (F-EAST1 and 2) were combined into a single population F-EAST.
[f] Due to small samples sizes (cc, $n < 9$) these sampling sites were excluded from further population genetic analyses.
[g] Nd = not determined.

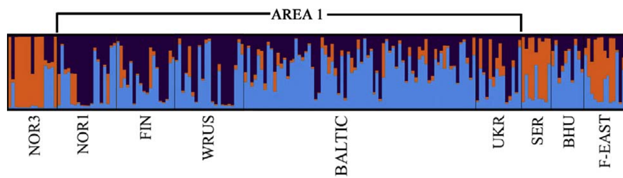

Fig. 3. STRUCTURE clustering of the *D. septosporum* cc dataset, optimal number of clusters (K = 3) by ln(Pr(X|K)). Every vertical column represents one haplotype. Different colours (light blue, purple and orange) represent the membership fraction of each haplotype to each cluster. Area 1 is based on information represented in Fig. 1. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

to be supported in the EST2 (both non-cc and cc datasets) and the F-EAST1 (cc dataset only) sampling sites.

3.5. Isolation by distance and clustering analysis

The Mantel test for isolation by distance among the seven populations of Europe revealed significant correlation between geographical distance and Nei's genetic distance ($R^2 = 0.517$, $p = 0.009$). No significant correlation ($R^2 = 0.056$, $p = 0.182$) was found when all nine Eurasian populations were included in the Mantel test.

The ln(Pr(X|K)) method of choosing the best number of STRUCTURE clusters indicated three clusters best describe the dataset (Fig. 3). None of the populations fell into a single defined cluster, instead, all were divided among two or three main clusters. Isolates from BALTIC, FIN, NOR1, UKR and WRUS (Area 1) were mainly placed into two clusters (blue and purple). In the Serbian, Russian Far East and Norwegian (NOR3) populations the orange cluster dominated, which did not dominate in any other analysed population (Fig. 3).

It is notable that none of the Bhutan isolates fell into the orange cluster, even though it was dominating in another Asian population F-EAST, i.e. the geographically closest sampled site. The STRUCTURE cluster composition of the Bhutan population was more similar to the northern European populations, particularly to the Finnish population, and was dominated by the blue cluster.

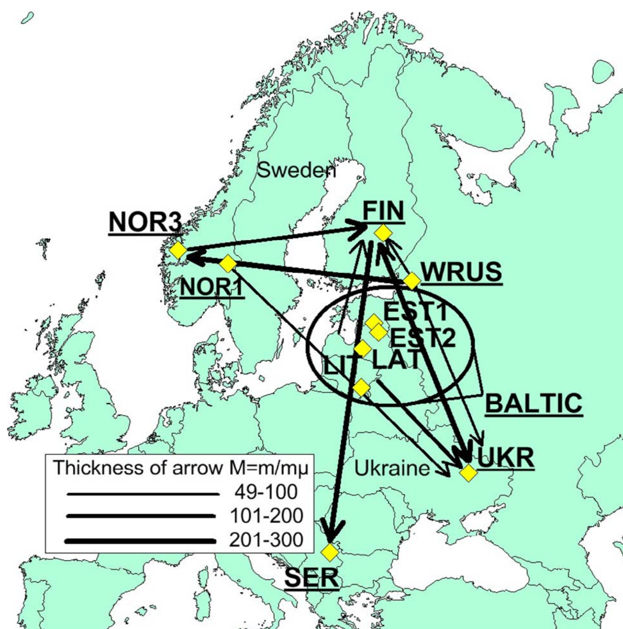

Fig. 4. Migration between European populations of *D. septosporum* (names underlined). The extent of migration increases with the thickness of arrow lines. Similar populations in Baltic countries (BALTIC) are marked by a circle.

3.6. Migration between populations of *D. septosporum*

Immigrants per generation (theta) varied considerably among population pairs (0.002–0.096 immigrants per generation; Table S3). The WRUS population of *D. septosporum* was the most frequent source of migration and UKR was the most frequent receiving (sink) area of gene flow from other populations in Eurasia (see Figs. 4 and 5).

Migration between different populations in Europe is a prominent feature (Fig. 4). In Asia, the most frequent destination area was Bhutan (Fig. 5). Migration analyses between the populations from Europe, Russian Far East and Bhutan revealed migration from Europe to Russian Far East and from Far East to Bhutan (Table S4 and Fig. 5).

4. Discussion

This is the first investigation analysing *D. septosporum* populations in the range of *Pinus* species of the Eurasian forest belt from the Atlantic to the Pacific Ocean. The most diverse populations were found in north-east Europe (Baltic countries, Finland, European Russia, Norway (NOR1) and Ukraine) that corresponds with the centre of the native range of *P. sylvestris* in Europe. This result, together with the lowest Nei genetic distances, low number of clones, high mean allelic and private allelic richness, and high mean haploid genetic diversity suggest that the fungus may be native to this area. Relationships between remote populations of *D. septosporum* in north-east Europe and Asia were detected by clustering analyses, because similar genetic patterns were found in Norway, Serbia and Russian Far East. In addition, migration analysis indicated that gene flow could have occurred from north-east European *D. septosporum* populations to the Russian Far East and Bhutan populations, however, no shared haplotypes were detected between the analysed populations of *D. septosporum* in Eurasia. Both mating type idiomorphs were found in more or less equal frequencies in almost all populations studied revealing evidence of sexual recombination of *D. septosporum* in northern Europe and Asia.

4.1. *Dothistroma septosporum* in north-east Europe

The occurrence of isolation by distance among the European populations of *D. septosporum* in this study suggests the pathogen has been present for considerable evolutionary time and supports the possibility that the fungus is native to the region. *Pinus sylvestris* is the sole native pine species in northern Europe and, notably, *D. septosporum* causes only mild symptoms on this tree species in the region (Drenkhan et al., 2016). This phenomenon is generally typical of hosts and pathogens that have experienced a long coexistence and thus coevolution (Ennos, 2001; Harrington and Wingfield, 1998). It is also worth noting that the genetic diversity of *P. sylvestris* is highest in northern and eastern Europe (Buchovska et al., 2013; Kärkkäinen, 2016; Naydenov et al., 2007), which broadly correlates with the area of highest genetic diversity of *D. septosporum* found in this study. In addition, the Baltic countries, Finland, European Russia and Ukraine are relatively close, geographically, to an area south-east of Moscow, where *P. sylvestris* survived during the last ice age in Europe (Buchovska et al., 2013). It is probable that the pathogen survived there together with the host (see Fig. 1). Taken together the results of this study agree with previous suggestions that European Scots pine could be the original historical host of *D. septosporum* (Drenkhan et al., 2013; Mullett et al., 2017; Perry et al., 2016; Piotrowska et al., 2017) and that *D. septosporum* could be native to Europe (Barnes et al., 2014; Drenkhan et al., 2013; Mullett et al., 2017; Piotrowska et al., 2017). From a practical point of view, understanding the origin of *D. septosporum,* could help mitigate the spread of the pathogen to new areas or the spread of potentially more aggressive genotypes, elsewhere.

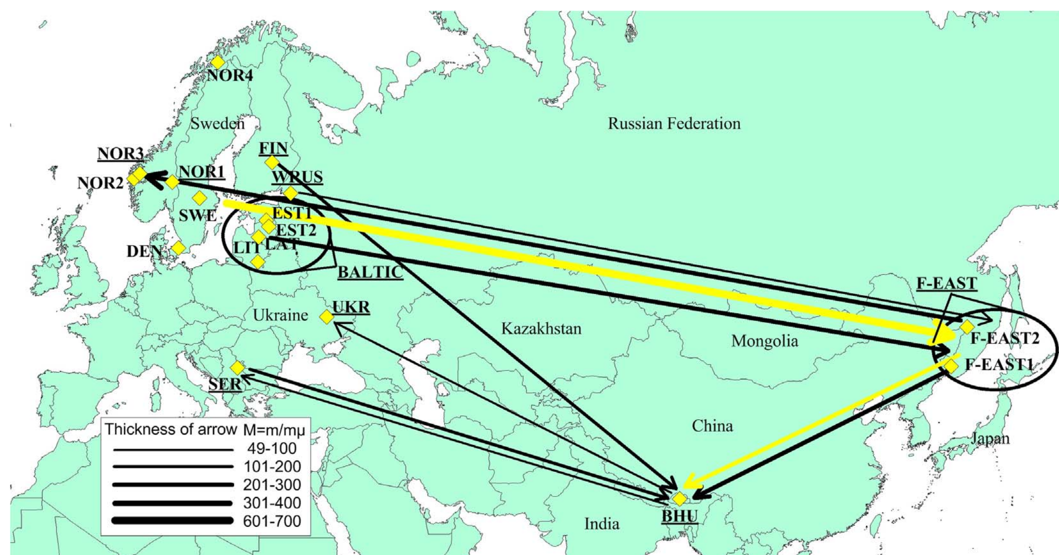

Fig. 5. Migration between *D. septosporum* populations in Europe and Far East Asia (underlined names). The extent of migration increases with the thickness of arrow lines. Similar populations in Baltic countries and in Russian Far East, respectively, were combined and shown as two single populations (marked with circles). Yellow arrows indicate migration from Europe (all populations merged) to Russian Far East and from Russian Far East to Bhutan. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4.2. Dothistroma septosporum in Asia

In eastern Asia (Russian Far East) molecular tools confirmed the presence of *D. septosporum* but showed no evidence of *D. pini* in Primorskiy Kray or Khabarovskiy Kray. Similarly, in south-central Asia (Bhutan) only *D. septosporum* has been recorded so far (Barnes et al., 2014). The Asian populations (Russian Far East and Bhutan) contained clones, showed low mean allelic and private allelic richness and relatively low mean haploid genetic diversity when compared to the north-east European populations. All analysed Asian populations were located outside of the native range of *P. sylvestris*. Seventeen Asian isolates of *D. septosporum* were isolated from hosts, which are non-native to the area (i.e. *P. sylvestris* and *P. radiata* D. Don.), although isolates (n = 10) were also obtained from native hosts (i.e. *P. densiflora* and *P. wallichiana* Jacks.). The populations included in this study do not support the hypothesis that Russian Far East or Bhutan could be the geographic origin of *D. septosporum* as suggested by Ivory (1994), however a much larger sample size of the Asian populations is needed to thoroughly investigate this.

Results from our study indicated that it is more likely that *D. septosporum* has been introduced to Russian Far East from Europe. This is because the genetic diversity is lower in Asia than Europe (Table 2) and migration analyses showed more significant gene flow from west to east (Tables S3, S4 and Fig. 5). No identical haplotypes were found between populations in north-east Europe and Asia and therefore, there is no evidence for direct movement of the pathogen. It remains unclear whether the introduction of the pathogen into Asia has occurred through anthropogenic means or via natural dispersal.

The two Asian populations (F-EAST and Bhutan) are clearly genetically distinct from each other as shown by Nei's genetic distance and clustering analyses (Fig. 3). Barnes et al. (2014) stated that the Bhutan population is distinct compared to other populations on a global scale. Natural dispersal of the pathogen from other populations in Europe or Asia to Bhutan seems unrealistic, because Bhutan is separated from the natural geographical range of the genus *Pinus* by a large mountainous area (see Fig. S1). It is interesting to note however, that in Bhutan, one of the host species from which *D. septosporum* isolates were collected was *P. radiata*. This is clearly an exotic host that was planted in this area and could possibly indicate pathways of anthropogenic movement of the pathogen. This hypothesis would, however, need to be further tested.

4.3. Potential spread of D. septosporum in Eurasia

No isolation by distance was detected when all European and Asian populations were tested. This may suggest that the gene flow of *D. septosporum* from north-east Europe to Russian Far East has been promoted by artificial, or human mediated, forces (e.g. trade of seedlings) not influenced by distance. However, it is more likely that the large geographical gap in sampling between the two regions has obscured any potential correlation between geographical and genetic distances. A natural pathway for dispersal from north-east Europe to Russian Far East exists via a continuous forest belt, containing various *Pinus* species from Europe to the coast of the Pacific Ocean (Fig. S1) and such a pathway was suggested by Ivory (1994). All *Pinus* species of this "pine belt" (*P. sylvestris, P. sibirica, P. densiflora, P. pumila,* and *P. koraiensis*) are known hosts of *D. septosporum* (Drenkhan et al., 2016). There are five documented records of DNB from this hypothetical fungal natural gene-flow bridge from Europe to Asia (http://arcgis.mendelu.cz/monitoring/) although the exact species of *Dothistroma* has not always been known (Drenkhan et al., 2016). Some non-native *P. sylvestris* stands and groups of ornamental trees exist in the Russian Far East (R. Drenkhan, unpublished data), but we were unable to find any data suggesting large-scale introductions of European pine species to this area (A. Bogacheva, personal comm.). Anthropogenic dispersal of trees in the opposite direction, i.e. from the Far East to Europe has occurred for more than a century (Andronov, 1953; Drenkhan et al., 2014; Eurostat, 2015; Koponen and Koponen, 1995; Plotnikova, 1971).

Gene flow from Europe or Russian Far East to Bhutan via natural dispersal pathways is unlikely due to the absence of any connecting belts of natural continuous pine forest between Bhutan and Europe or the Far East (Fig. S1). A geographical gap, between 26–28°N and 91–93°E of about 100–150 km, contains no native *Pinus* sp., however the presence of other susceptible conifer species is unknown.

Some seed or seedling transmission between Europe and Bhutan at the end of 19th century and in the beginning of the 20th century has been documented (Monumental trees, 2017). Furthermore, it has been reported that seed lots contaminated with needle debris can transmit the fungus over long distances (EPPO; Gibson, 1974).

4.4. The unique populations of D. septosporum in northern Europe

The AMOVA revealed that the NOR3 population is significantly

different from NOR2 ($p < 0.05$; see Table S1), irrespective of the short geographic distance between the sampling sites (*ca.* 60 km). This is in stark contrast to the large geographical distances between sampling sites grouped by the AMOVA into single populations, such as the BALTIC and F-EAST populations with maximal distances between sampling sites of *ca.* 400 and 650 km, respectively. An explanation for the divergence between the two Norwegian populations may be that they are separated by high mountains, which may indicate that these natural barriers have hindered gene flow between fungal populations (Rogers and Rogers, 2012). Isolates from the northernmost known sampling site of *D. septosporum* in the world, i.e. from beyond the Arctic Circle in northern Norway (NOR4) had only one mating type *MAT1-2* (Table 4). This may indicate that the population has been recently introduced into this harsh environment. The presence of only a single mating type typically indicates an introduction event, such as what is seen in Chile, Ecuador, Australia and New Zealand (Barnes et al., 2014).

Clustering analyses showed that NOR3 is distinct from the other Fennoscandian populations (Fig. 3). It is known that some conifer trees (including *P. sylvestris*) survived the last glaciation event in Scandinavian refugia in central and north-western Norway (Parducci et al., 2012). We may speculate that the different genetic pattern of *D. septosporum* in the Norwegian population NOR3 could partly represent survival over the glaciation period in Norway. Perhaps more likely is that this population is a more recent introduction from another, unsampled, population. Alternatively, the clustering patterns might hint towards connections between northern Europe and Russian Far East or East Asia, perhaps via the plant or timber trade.

This study strengthens the hypothesis that the native range of *D. septosporum* could be in the Scots pine forests of northern Europe, primarily because the most diverse and genetically similar populations were found in the Baltic states, Finland, western Russia, Norway (NOR1) and Ukraine. In addition, the results show that some of the European populations are highly dissimilar to other analysed European (NOR3 and SER) populations. However, the gene flow of *D. septosporum* from northern Europe to Far East Russia was surprisingly higher than in the opposite direction, suggesting spread of the pathogen from northern Europe to Far East Russia. It is important to note that the results of the Asian populations might be influenced by sampling bias due to the small sample size of the Asian population. Thus, more isolates from various parts of Asia should be collected and analysed to enable a detailed comparison of the diversity of Asian and European *D. septosporum* populations, and to test the above derived hypothesis on the origin of the pathogen in northern Europe.

Authors contributions

R.D., M.S.M. and K.A. designed the research. R.D., K.A., H.S., M.S.M., I.B., M.M.M., M.V., A.K., S.M., K.D., N.K., R.D.P., D.L.M., K.L. and H.M. collected samples. K.A. and M.M. analysed the data. All authors wrote the manuscript.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.fgb.2017.12.001.

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